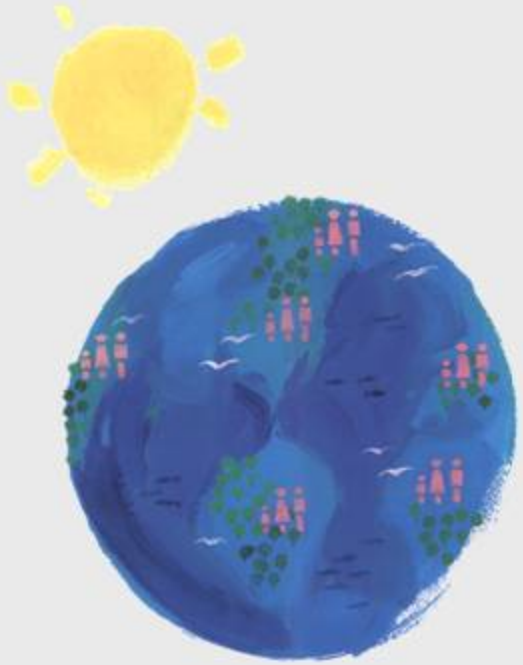
NICEATM

National Toxicology Program
Interagency Center for the Evaluation of Alternative Toxicological Methods

ICCVAM

Interagency Coordinating Committee on the Validation of Alternative Methods



Overview of the LLNA Independent Scientific Peer Review Panel Report

Michael Luster, Ph.D.
Chair, ICCVAM LLNA Peer Review Panel

June 26, 2009
SACATM Meeting
Arlington, VA

National Toxicology Program

NIEHS

National Institutes of Health

Department of Health & Human Services

Peer Panel Meeting – April 28-29, 2009

- ICCVAM and NICEATM convened an international independent scientific peer review panel to evaluate three modified nonradiolabeled versions and new applications for the murine local lymph node assay (LLNA)

Peer Review Panelists

- **Nathalie Alépée, Ph.D.**
  L'Oreal Research and Development, France
- **Anne Marie Api, Ph.D.**
  Research Institute for Fragrance Materials, USA
- **Nancy Flournoy, M.S., Ph.D.**
  University of Missouri, USA
- **Dagmar Jírová, M.D., Ph.D.**
  National Institute of Public Health, Czech Republic
- **David Lovell, Ph.D.**
  University of Surrey, United Kingdom
- **Michael Luster, Ph.D.**
  (Panel Chair)
  Senior Consultant to NIOSH Health Effects Laboratory, USA
- **Howard Maibach, M.D.**
  Univ. California - San Francisco, USA
- **Michael Olson, Ph.D.**
  (Evaluation Group A Chair)
  GlaxoSmithKline, USA
- **Raymond Pieters, Ph.D.**
  Utrecht University, The Netherlands
- **Jean Regal, Ph.D.**
  University of Minnesota, USA
- **Jonathan Richmond, MB ChB, FRCSEd**
  Animals Scientific Procedures Division, United Kingdom
- **Peter Theran, V.M.D.**
  Massachusetts Society for the Protection of Cruelty to Animals, USA
- **Stephen Ullrich, Ph.D.**
  (Evaluation Group B Co-Chair)
  MD Anderson Cancer Center, USA
- **Michael Woolhiser, Ph.D.**
  (Evaluation Group B Co-Chair)
  Dow Chemical Company, USA
- **Takahiko Yoshida, M.D., Ph.D.**
  Asahikawa Medical College, Japan

Peer Panel Report – June 1, 2009

- These are abbreviated highlights of the final Independent Scientific Peer Review Panel report
  - The final report should be consulted for a detailed description of the Panel's conclusions and recommendations
    - See http://iccvam.niehs.nih.gov/methods/immunotox/llna.htm

ICCVAM Charges to the Peer Panel

- Review the ICCVAM Revised Draft Background Review Documents (BRDs) for completeness, and identify any errors or omissions in the BRDs (LLNA: DA; LLNA: BrdU-ELISA; LLNA: BrdU-FC)
- Review the ICCVAM Revised Draft Applicability Domain Addendum for completeness, and identify any errors or omissions
- Evaluate the information in the revised draft documents to determine the extent to which each of the applicable criteria for validation and acceptance of toxicological test methods (ICCVAM Submission Guidelines 2003) have been appropriately addressed
- Consider the ICCVAM revised draft test method recommendations for the following, and comment on the extent to which they are supported by the information provided in the revised draft documents:
  - Proposed test method usefulness and limitations
  - Proposed recommended standardized protocols
  - Proposed test method performance standards
  - Proposed future studies

LLNA Modifications and Applications Evaluated

- Three modified versions of the LLNA not requiring radiolabeling:
  - LLNA: DA (LLNA: Daicel Adenosine Triphosphate)
  - LLNA: BrdU-ELISA (LLNA: Bromodeoxyuridine Detected by ELISA)
  - LLNA: BrdU-FC (LLNA: Bromodeoxyuridine Detected by Flow Cytometry)
- Application of the LLNA for evaluating pesticide formulations and other products, metals, and substances in aqueous solutions

Modified LLNA Protocol – Nonradiolabeled LLNA: DA (1)

- The Panel concluded that the available data and test method performance support the use of the LLNA: DA to identify substances as potential skin sensitizers and nonsensitizers, with certain limitations (see next slide)
- They concurred with ICCVAM that, based on the current validation database, multiple stimulation indices should be used as decision criteria to identify sensitizers and nonsensitizers for this method
  - SI ≥ 2.5 for sensitizers
  - SI ≤ 1.7 for nonsensitizers

Modified LLNA Protocol – Nonradiolabeled LLNA: DA (2)

- The Panel noted that the limitation of this test method when using the proposed multiple decision criteria is the indeterminate classification of substances that fall in the range of SI values for which a classification decision is not definitive (1.7 < SI < 2.5)
- For such results, the Panel recommended that:
  - Users should carefully interpret the results in an integrated decision strategy in conjunction with all available and relevant information to determine if there is adequate information for a definitive sensitization hazard classification or if additional testing is necessary
  - Available and relevant information may include dose response information, QSAR information, statistical analyses of the difference between treated and vehicle control groups, peptide-binding activity, molecular weight, results from related chemicals, and other testing data

Modified LLNA Protocol – Nonradiolabeled LLNA: DA (3)

- Even with these limitations, the LLNA: DA provides opportunities to reduce animal usage (e.g., use of guinea pigs) in those regions in which guinea pig tests rather than the traditional LLNA are performed because radioisotope use is not permitted
- In addition, the use of two decision criteria allows for a more definitive identification of sensitizers and nonsensitizers, which also provides animal welfare benefits by reducing further tests that might be required in instances where the hazard classification of a substance is not as clear

Modified LLNA Protocol – Nonradiolabeled LLNA: BrdU-ELISA (1)

- The Panel concluded that the available data and test method performance support the use of the LLNA: BrdU-ELISA to identify substances as potential skin sensitizers and nonsensitizers, with certain limitations (see next slide)
- They agreed with ICCVAM that, based on the current validation database, multiple stimulation indices should be used as decision criteria to identify sensitizers and nonsensitizers for this method
  - SI ≥ 2.0 for sensitizers
  - SI < 1.3 for nonsensitizers

Modified LLNA Protocol – Nonradiolabeled LLNA: BrdU-ELISA (2)

- The Panel noted that the limitation of this test method when using the proposed multiple decision criteria is the indeterminate classification of substances that fall in the range of SI values for which a classification decision is not definitive ($1.3 \leq SI < 2.0$)
- For such results, the Panel recommended that:
  - Users should carefully interpret the results in an integrated decision strategy in conjunction with all available and relevant information to determine if there is adequate information for a definitive sensitization hazard classification or if additional testing is necessary
  - Available and relevant information may include dose response, QSAR information, statistical analyses of the differences between treated and vehicle control groups, peptide-binding activity, molecular weight, results from related chemicals, and other testing data

Modified LLNA Protocol – Nonradiolabeled LLNA: BrdU-ELISA (3)

- Even with these limitations, the LLNA: BrdU-ELISA provides opportunities to reduce animal usage (e.g., use of guinea pigs) in those regions in which guinea pig tests rather than the traditional LLNA are performed because radioisotope use is not permitted
- In addition, the use of two decision criteria allows for a more definitive identification of sensitizers and nonsensitizers, which also provides animal welfare benefits by reducing further tests that might be required in instances where the hazard classification of a substance is not as clear

Modified LLNA Protocol – Nonradiolabeled LLNA: BrdU-FC (1)

- The Panel concluded that the database of more than 45 representative test substances yielded adequate accuracy based on results from one laboratory, and that intralaboratory reproducibility had also been adequately demonstrated
- However, the Panel agreed with the ICCVAM proposal to defer a formal recommendation on the validity of the LLNA: BrdU-FC until the following conditions are met:
  - An independent audit of all data supporting the analysis has been conducted by the NTP; and
  - Transferability has been demonstrated in an interlaboratory validation study

Modified LLNA Protocol – Nonradiolabeled LLNA: BrdU-FC (2)

- The Panel recommended that ICCVAM should work with NICEATM to support and facilitate the independent audit and interlaboratory validation study
- The Panel recommended that upon completion of these tasks and determination of satisfactory data quality and interlaboratory reproducibility, that the LLNA: BrdU-FC could be considered scientifically valid and useful for regulatory safety testing

Modified LLNA Nonradiolabeled Protocols – General Conclusions

- The Panel concluded that all three of the nonradiolabeled LLNA protocols are mechanistically and functionally similar to the traditional LLNA, and therefore do not require separate test method performance standards
- The Panel considered that an emphasis should be made to include ear swelling measurements and immunophenotypic markers as an indicator of irritation for the traditional LLNA and for any modified LLNA test methods

LLNA Applicability Domain (1)

- With regard to the applicability of the LLNA for testing pesticide formulations and other products (e.g., natural complex substances, dyes) and substances in aqueous solutions, the Panel concluded:
  - Any material should be a candidate for testing in the LLNA unless there are unique physicochemical properties associated with the class of test materials that might affect its ability to interact with the immune processes
  - Therefore, the LLNA should be considered applicable to pesticide formulations and other products, and substances in aqueous solutions unless there is a biologically-based rationale for exclusion
- No metal substances were added to the database

LLNA Applicability Domain (2)

- The Panel expressed a strong desire to avoid revalidation of the LLNA for new classes/types of test substances unless there is a biologically-based rationale
  - For new classes of test materials (e.g., nanomaterials), an integrated assessment of relevant available information should be conducted
    - This includes computer-assisted structure-activity relationships, prediction/measurement of biotransformation to potential reactive species, and possibly peptide, protein, or lipid binding
- The Panel agreed that if any variant of the LLNA is validated for use to test novel classes, then the findings should be relevant to the family of validated LLNA tests

Questions to SACATM (1)

1. Do you have any comments on the Panel's conclusions and recommendations on the three revised draft ICCVAM BRDs for the nonradioactive LLNA assays in regard to their completeness and any identified errors or omissions?
2. Do you have any comments on the Panel's conclusions and recommendations in terms of the extent to which each of ICCVAM's applicable criteria for validation and acceptance of alternative test methods have been addressed appropriately in each revised draft test method BRD or the revised draft BRD appendices?

Questions to SACATM (2)

3. Do you have any comments on the Panel's comments, conclusions or recommendations for the LLNA methods and applications regarding:
   a. their usefulness and limitations?
   b. the recommended test method protocols?
   c. test method performance standards?
   d. the proposed additional studies?

4. Do you have any comments on the Panel's comments, conclusions or recommendations regarding the ICCVAM revised draft LLNA Applicability Domain for:
   a. pesticide formulations and other products?
   b. metals?
   c. aqueous solutions?